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OBSERVATIONS ON THE THERMOSTABILITY OF OROSOMUCOID

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SUMMARY

Orosomucoid is shown to have no special resistance to thermal denaturation, contrary to previous thought. Earlier conclusions probably arose from the great solubility of the molecule, even at elevated temperature. Under otherwise physiological conditions, orosomucoid begins denaturing at ~46°C with a T_m of 62°C. The denaturation is reversible in the absence of aggregation. The terminal sialic acid residues of the carbohydrate sidechains appear to contribute to the overall stability of the molecule in a limited and negative way.

INTRODUCTION

Orosomucoid (α_1-acid glycoprotein) is an acute phase serum glycoprotein of unknown function. It has been extensively studied both physically (1,2) and chemically (1,2), and both the polypeptide (3) and carbohydrate (4) chain sequences have been determined. It is currently the subject of intense study and has gained much favor as a model ligand for the hepatic asialoglycoprotein receptor (5). It has been implicated amongst others, in such physiological activities as collagen fibril formation (6) immunosuppressive action (7), the binding of a large number of basic drugs (8), and steroid hormones (9), and the sequestration of a cofactor in the lipoprotein lipase reaction (10). These studies have all stood on the widely held view that orosomucoid is extremely thermostable, up to temperatures of 100°C. In other work we have found that the ligand binding properties of orosomucoid fall off dramatically above about 46°C (11) and that

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polymers could be formed above this temperature (12). These observation suggested that orosomucoid is not thermostable and that inappropriate criteria had been used previously to assess this property. A knowledge of the degree of instability of the molecule is especially important in any study which makes presumptions of the native state of the sample, for example ligand binding.

MATERIALS AND METHODS

Orosomucoid was prepared from nephrotic urine as previously reported (12), with additional chromatographic steps on CM-32 (Whatman) eluting with 0.006 M citrate + 0.0054 M phosphate pH 4.0, and DEAE-32 (Whatman) eluting with 0.02 M Tris-HCl + 0.2 M NaCl pH 8.6 (13). Delipidation of the product was according to Ganguly et al. (14). Orosomucoid prepared in this manner was homogeneous by SDS gel electrophoresis, analytical gel chromatography and immuno-electrophoresis. All other reagents were reagent grade or better.

Differential scanning calorimetry was performed on an MC-1 (Microcal Inc., Amherst, Mass.), using a ramp of 1°C/min. Known equal volumes of deaerated sample and blank were delivered to the precooled twin platinum cells of the calorimeter with a calibrated microsyringe, and allowed to temperature equilibrate. Scans were in the range of 0°-90°C.

Data analysis of endotherms followed that of Jackson and Brandts (15).

RESULTS AND DISCUSSION

Fig. 1a shows a typical endotherm for defatted orosomucoid in phosphate buffered saline at pH 7.4. A slightly asymmetric single endotherm was obtained with a T_m of 62°C and ΔH (cal) of 120 kcal/mole. Rescanning of a sample heated to T_m and cooled gave an endotherm essentially indistinguishable from that in Fig. 1a. Gel chromatography of the product of heating under these conditions showed that a small amount (<5%) of polymer was formed. Thus, until a value is available for the heat of polymerization, ΔH (cal) is probably biased low by a small amount since aggregation is generally an exothermic process. The ratio of ΔH (cal) to ΔH (Van't Hoff) may give an indication as to whether or not the native to denatured transition is a two-state process (16,17). For the transition shown, the ratio is 1.25 indicating the

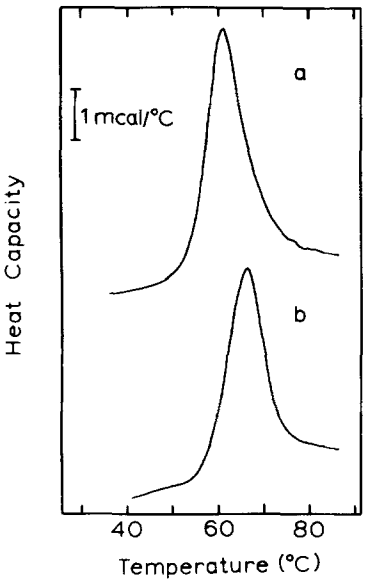

Figure 1. Differential scanning calorimetric endotherms for orosomucoid. (a) Defatted orosomucoid (3.305×10^{-4} M) in phosphate buffered saline pH 7.4 (0.01 M phosphate + 0.14 M NaCl), scanned at 1°C/min. (b) Defatted asialoorosomucoid (2.517×10^{-4} M), conditions as for a.

existence of intermediates during unfolding. An exothermic aggregation would tend to mask this effect, and indeed, under conditions of extensive polymerization (citrate-phosphate pH 4.0), the ratio takes the value of 0.4. Stellwagen and Wilgus (18) have related empirically protein thermostability to the area of the molecule exposed to solvent. Orosomucoid contains five polysaccharide chains which comprise 40% of the weight of the molecule. If the protein portion of the molecule is analyzed according to Stellwagen and Wilgus, it falls almost exactly on the least squares line they find relating T_m to exposed area for monomeric proteins, suggesting that the carbohydrate plays little role in thermostability, and the polypeptide chain has no especially thermostable properties. Aubert and Loucheux-Lefebvre (19) have used the Lim (20) and Chou and Fasman (21) predictions to evaluate the secondary structure of the protein portion of orosomucoid and also compare the theoretical and experimental circular dichroic spectra. Allowing for

the uncertainties in the circular dichroic simulations, they conclude that the carbohydrate moieties do not produce any perturbation of the protein conformation. Kawahara et al. (22) also reported no involvement of the sialic acid residues in the protein secondary structure, as measured by circular dichroism. That a change does occur, however, is suggested by the observation that sialic acid free orosomucoid has a slightly higher association constant for progesterone than when the sialic acid is present (9). We find that the sialic acid portion of the carbohydrate chains does contribute to thermostability in a negative sense since its removal increases the cooperativity, ΔH (cal) (140 kcal/mole) and T_m (66°C) for the transition (Fig. 1b). Removal of the sialic acid, then, appears to stabilize the protein, probably by relaxing the constraints due to mutual repulsion of the negatively charged sialic acid residues at pH 7.4. The inability of CD to detect the changes reflects, perhaps, an overall tightening of the molecule, rather than a localized one. Gel chromatography of the product of heating the asialoorosomucoid generally showed a large conversion to polymers. Direct comparison of the denaturation heats is therefore of dubious value, but more importantly, this shows that polymerization is controlled by the large excess negative charge which acts to hinder the approach of orosomucoid molecules to each other. In other work (13) we have been able to show the formation of small amounts of polymers in orosomucoid solutions at 37°C and below at pH 7.4. It then becomes of interest to conjecture on the physical form of asialoorosomucoid which is bound by the hepatic asialoglycoprotein receptor (5).

CONCLUSION

In physiological solvents, orosomucoid demonstrates a thermostability to be expected of a polypeptide chain of its size. Earlier suggestions of thermostability probably derived from the extreme

solubility of the molecule at high temperatures due to the high carbohydrate content.

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